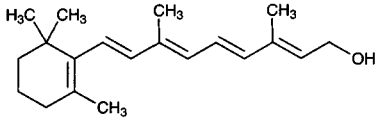
REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Vitamin A



Molecular formula: $C_{20}H_{30}O$
Molecular weight: 286.46
CAS Registry No.: 68-26-8
Merck Index: 10150

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 2 mL EtOH + 10 mL hexane, mix for 30 s, centrifuge at 3000 rpm for 5 min, store the hexane layer at 15°, repeat the extraction with 10 mL hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute with 200 µL isopropanol, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm TSKgel ODS-80Ts
Mobile phase: Gradient. EtOH:water 80:20 for 11.5 min then 87:13 (step gradient)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 460

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Extracted: vitamin E (F ex 298 em 325)

KEY WORDS
serum

REFERENCE
Moriyama,H.; Yamasaki,H.; Masumoto,S.; Adachi,K.; Katsura,N.; Onimaru,T. Rapid determination of vitamins A and E in serum with surfactant as a diluent by column-switching high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *798*, 125–130.

SAMPLE
Matrix: blood
Sample preparation: Dilute 100 µL serum with 900 µL 12.62 mg/mL pyrogallol in EtOH, filter (450 µm cellulose disk), cool at 15° in the autosampler, inject a 300 µL aliquot onto column A and elute to waste with mobile phase A. After 3 min backflush the contents of column A onto column B with mobile phase B, after another 1 min remove column A from the circuit. Elute column B with mobile phase B for another 7.5 min then elute with mobile phase C. Monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4.6 13 µm TSK BSA-80Ts; B 15 × 3.2 5 µm TSK ODS-80Ts + 150 × 4.6 5 µm TSKgel ODS-80Ts
Mobile phase: A 200 mM sodium dodecyl sulfate solution:EtOH 70:30 containing 200 mM ethylenediaminetetraacetic acid 4 sodium salt and 0.3% phosphoric acid; B EtOH:water 80:20; C EtOH:water 87:13
Column temperature: 40

Flow rate: A 1.5; B 1; C 1
Injection volume: 300
Detector: F ex 340 em 460

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 1.67 IU/dL

OTHER SUBSTANCES
Extracted: vitamin E (F ex 298 em 325)

KEY WORDS
serum; column-switching

REFERENCE
Moriyama,H.; Yamasaki,H.; Masumoto,S.; Adachi,K.; Katsura,N.; Onimaru,T. Rapid determination of vitamins A and E in serum with surfactant as a diluent by column-switching high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *798*, 125–130.

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL serum with 200 μL MeCN and 10 μL 20 mM ascorbic acid, centrifuge at 16000 g for 5 min. Mix the supernatant with 200 μL water, inject a 2 μL aliquot. (Protect all solutions from light.)

HPLC VARIABLES
Column: 50 × 0.18 3 μm ODS-AQ (YMC, Wilmington, NC) (The separation capillary column was formed from fused-silica capillaries (Polymicro Technologies, Phoenix) by inserting a small 50 μm I.D. capillary ca. 15 mm into a larger 180 μm I.D. capillary and fixed by applying epoxy (No. 353ND, Epoxy Technology, Billerica MA). A glass filter paper frit (Whatman GF/A) was inserted into the larger capillary and forced against the smaller capillary with a stream of isopropanol. The stationary phase was suspended in 3 mL isopropanol and pumped into the larger capillary until a 50 mm bed was formed. The larger and smaller diameter capillaries extended no more than 100 and 16 mm from the frit, respectively.)
Mobile phase: MeCN:MeOH:water 65:2.5:32.5 containing 1% tetrabutylammonium perchlorate, adjusted to pH 5.0 with acetic acid and 174 mM sodium acetate
Flow rate: 0.004
Injection volume: 2
Detector: E, carbon-fiber working electrode +900 mV, Ag/AgCl reference electrode (details of preparation in paper)

CHROMATOGRAM
Retention time: 11
Limit of detection: 38 pg/mL
Limit of quantitation: 70 fmol

OTHER SUBSTANCES
Extracted: isotretinoin, retinaldehyde, tretinoin

KEY WORDS
cow; serum; capillary HPLC

REFERENCE
Hagen,J.J.; Washco,K.A.; Monnig,C.A. Determination of retinoids by reversed-phase capillary liquid chromatography with amperometric electrochemical detection, *J.Chromatogr.B*, **1996**, *677*, 225–231.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 837 ng/mL retinyl acetate in EtOH and 400 μL EtOH to 500 μL plasma, vortex for 30 s, add 2 mL hexane, vortex for 30 s, centrifuge at 1500 rpm for 5 min. Remove the upper hexane layer, add 2 mL hexane to the lower layer, reextract. Evaporate

the combined hexane layers to dryness under nitrogen at 40°, reconstitute the residue in 200 μL EtOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm 100 Å pore size Supelguard column (Supelco)
Column: 250 × 4.6 5 μm 100 Å pore size Suplex pKb-100 RP (Supelco)
Mobile phase: MeOH:MTBE:water 80:20:5
Column temperature: 0
Flow rate: 0.8
Injection volume: 50
Detector: UV 328

CHROMATOGRAM
Retention time: 6.7
Internal standard: retinyl acetate (7.8)

KEY WORDS
plasma

REFERENCE
Lane,J.R.; Webb,L.W.; Acuff,R.V. Concurrent liquid chromatographic separation and photodiode array detection of retinol, tocopherols, all-trans-α-carotene, all-trans-β-carotene and the mono-cis isomers of β-carotene in extracts of human plasma, *J.Chromatogr.A*, **1997**, *787*, 111–118.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge 200 μL serum, add 200 μL IS and 200 μL EtOH, mix on orbital shaker for 5 min, add 200 μL water and 500 μL hexane, mix for 10 min, centrifuge at 2000 g for 10 min at 17°, remove 300 μL upper organic layer. Re-extract with 300 μL hexane, mix for 10 min, centrifuge at 4000 g for 10 min at 17°, remove 300 μL upper organic layer. Combine the organic layers, and evaporate them to dryness under vacuum in 15 min. Reconstitute the residue with 300 μL MeOH:EtOH:hexane 88:10:2, vortex for 10 min, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Adsorbosphere HS C18 + 150 × 4.6 3 μm Adsorbosphere HS C18 in series
Mobile phase: Gradient. A was MeCN:MeOH 60:40 containing 0.05% acetic acid. B was MeCN:MeCN:dichloromethane 45.6:30.4:24 containing 0.04% acetic acid. A:B 100:0 for 7 min then 0:100 for 10.4 min (step gradient), re-equilibrate at initial conditions for 5.6 min.
Column temperature: 37
Flow rate: 0.9
Injection volume: 40
Detector: UV 325

CHROMATOGRAM
Retention time: 5.7
Internal standard: tocol (UV 292) (10.1), echinenone (UV 450) (12.8)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: canthaxanthine (UV 473), α-carotene (UV 450), β-carotene (UV 450), β-cryptoxanthine (UV 450), lutein (UV 450), lycopene (UV 473), vitamin E (UV 292), zeaxanthin (UV 450), nonidentified carotenoids

KEY WORDS
serum

REFERENCE
Steghens,J.-P.; van Kappel,A.L.; Riboli,E.; Collombel,C. Simultaneous measurement of seven carotenoids, retinol and α-tocopherol in serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *694*, 71–81.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 5% perchloric acid, mix rapidly, add 500 μL ethyl acetate, mix for 60-90 s, centrifuge at 13000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: Reversed-phase C18 (Waters or Bio-Rad)
Mobile phase: MeCN:1% ammonium acetate 75:25
Flow rate: 2.5
Detector: UV 340

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: isotretinoin, tretinoin

KEY WORDS
plasma; pharmacokinetics; protect from light

REFERENCE
Davis,T.P.; Peng,Y.-M.; Goodman,G.E.; Alberts,D.S. HPLC, MS, and pharmacokinetics of melphalan, bisantrene and 13-cis retinoic acid, *J.Chromatogr.Sci.*, **1982**, *20*, 511–516.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 5% perchloric acid, vortex for 30 s, add 500 μL ethyl acetate, whirl for 1 min, centrifuge at 13000 g for 1 min, inject a 50 μL aliquot of the organic layer.

HPLC VARIABLES
Guard column: present but not specified
Column: 250 × 4.6 5 μm Ultrasphere ODS + 300 × 4 10 μm μBondapak in series
Mobile phase: MeCN:1% ammonium acetate 95:5
Flow rate: 2.5
Injection volume: 50
Detector: UV 340, UV 365

CHROMATOGRAM
Retention time: 5.3
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: retinoic acid

KEY WORDS
protect from light; plasma

REFERENCE
Peng,Y.-M.; Xu,M.-J.; Alberts,D.S. Analysis and stability of retinol in plasma, *J.Natl.Cancer Inst.*, **1987**, *78*, 95–99.

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL 0.5 μg/mL retinyl acetate , 1 μg/mL retinyl palmitate, and 25 μg/mL α-tocopheryl acetate in EtOH to 1 mL serum or plasma while continuously vortexing, add 3 mL hexane, vortex for 2 min, centrifuge at 2500 g for 2 min, remove the upper phase, add 2 mL hexane to the lower layer, repeat extraction. Combine the upper layers and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 40 μL aliquot,

HPLC VARIABLES
Guard column: C18 (Waters)
Column: 5 μm Biophase ODS C18 (Bioanalytical Systems)
Mobile phase: MeCN:chloroform:isopropanol:water 78:16:3.5:2.5
Flow rate: 2
Injection volume: 40
Detector: UV 460 (UV 292 for tocopherol)

CHROMATOGRAM
Retention time: 2.60
Internal standard: retinyl acetate (3.07), retinyl palmitate (18.66), α-tocopheryl acetate (8.33)

OTHER SUBSTANCES
Extracted: β-carotene, vitamin E (α-tocopherol), gamma-tocopherol, α-carotene, lycopene, cryptoxanthin

KEY WORDS
serum; plasma

REFERENCE
Kaplan,L.A.; Miller,J.A.; Stein,E.A.; Stampfer,M.J. Simultaneous, high-performance liquid chromatographic analysis of retinol, tocopherols, lycopene, and α- and β-carotene in serum and plasma, *Methods Enzymol.*, **1990**, *189*, 155–167.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Serum + 25 μL 80 μg/mL tocol in EtOH + 250 μL 20 μg/mL BHT (butylated hydroxytoluene) in EtOH + 1.5 mL hexane, vortex for 1 min, remove 1 mL of upper layer, add 500 μL hexane, vortex for 1 min, remove 300 μL of upper layer. Combine the hexane extracts, evaporate to dryness under a stream of inert gas. Reconstitute in 250 μL 20 μg/mL BHT in EtOH, sonicate, centrifuge if necessary, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 201TP54 (wide pore, polymerically bonded C18)
Mobile phase: Gradient. A was MeOH:n-butanol:water 75:10:15 containing 50 mM ammonium acetate, pH 5.5. B was MeOH:n-butanol:water 88:10:2 containing 50 mM ammonium acetate, pH 5.5. A:B 100:0 for 3 min, to 0:100 over 15 min, maintain at 0:100 for 17 min
Injection volume: 25
Detector: UV 325 for 7 min, UV 295 for 13 min, UV 450 for 14 min or E, glassy carbon electrode, Ag/AgCl reference electrode +1050 mV for retinol, +900 mV for tocol, +750 mV for α-tocopherol, +700 mV for β-carotene

CHROMATOGRAM
Retention time: 5
Internal standard: tocol (13)
Limit of detection: 4.1 μg/mL (E), 6 μg/mL (UV)

OTHER SUBSTANCES
Extracted: β-carotene, vitamin E (α-tocopherol), gamma-tocopherol, lutein, zeaxanthin, cryptoxanthin, α-carotene, 9-cis-β-carotene

KEY WORDS
serum

REFERENCE
MacCrehan,W.A. Determination of retinol, α-tocopherol, and β-carotene in serum by liquid chromatography, *Methods Enzymol.*, **1990**, *189*, 172–181.

SAMPLE
Matrix: blood

Sample preparation: 200 μL Serum + 100 μL EtOH + 100 μL α-tocopheryl acetate in EtOH, vortex for 5 s, add 500 μL hexane, vortex for 2 min, centrifuge at 700 g for 5 min. Remove 250 μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, mix for 2 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm Spheri-10 RP18
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:dichloromethane:MeOH 70:20:10
Flow rate: 1.2
Injection volume: 50
Detector: UV 325 for 3.5 min, UV 291 for 4.5 min, UV 450 for 6 min

CHROMATOGRAM
Retention time: 2.31
Internal standard: α-tocopheryl acetate (6.30)
Limit of detection: 16 nM

OTHER SUBSTANCES
Extracted: beta carotene, vitamin E

KEY WORDS
protect from light; serum

REFERENCE
Arnaud,J.; Fortis,I.; Blachier,S.; Kia,D.; Favier,A. Simultaneous determination of retinol, α-tocopherol and β-carotene in serum by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *572*, 103–116.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma or serum + 100 μL 6 μg/mL 9-methylanthracene in MeOH + 1.5 mL MeCN + 100 μL 100 mM perchloric acid, flush headspace of vial with argon, vortex, centrifuge, inject a 50 μL aliquot of the supernatant. Sonicate serum, solvents, and mobile phase under vacuum before use. Use low actinic glassware and yellow light.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C18
Mobile phase: MeCN:0.5% acetic acid 85:15 containing 0.05% sodium hexanesulfonate
Flow rate: 2
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 9
Internal standard: 9-methylanthracene (5)
Limit of detection: 12 ng/mL

OTHER SUBSTANCES
Simultaneous: 13-cis-retinoic acid, all-trans-retinoic acid, 4-oxo-13-cis-retinoic acid

KEY WORDS
plasma; serum

REFERENCE
Gadde,R.R.; Burton,F.W. Simple reversed-phase high-performance liquid chromatographic method for 13-cis-retinoic acid in serum, *J.Chromatogr.*, **1992**, *593*, 41–46.

SAMPLE
Matrix: blood

Sample preparation: 2.5 mL Plasma + 2.5 mL 18 ng/mL IS1 and 10 ng/mL IS2 in EtOH, shake vigorously for 20 s, centrifuge at 1200 g for 5 min, add 5 mL diethyl ether, shake vigorously, centrifuge for 5 min, extract twice more with 5 mL ether. Combine ether layers, wash with 15 mL 5% NaCl, dry over sodium sulfate, evaporate to dryness under vacuum at 35°. Dissolve residue in 1-2 mL dichloromethane, filter (0.45 μm). Evaporate to dryness under a stream of nitrogen, make up to 100 μL with MeCN:MeOH:dichloromethane:hexane 45:10:22.5:22.5, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Spheri-5-C18 (Brownlee)
Column: 250 × 4.6 5 μm Microsorb C18 (Rainin)
Mobile phase: Gradient. MeCN:MeOH:dichloromethane:hexane 85:10:2.5:2.5 for 10 min then to 45:10:22.5:22.5 over 30 min, re-equilibrate for 15 min
Flow rate: 0.7
Injection volume: 20
Detector: UV 325

CHROMATOGRAM

Retention time: 7
Internal standard: IS1 ethyl β-apo-8'-carotenate (18), IS2 (3R)-8'-apo-β-carotene-3,8'-diol (5)

OTHER SUBSTANCES

Extracted: carotenoids, β-carotene, vitamin E (α-tocopherol)

KEY WORDS

plasma; handle under yellow lights

REFERENCE

Khachik,F.; Beecher,G.R.; Goli,M.B.; Lusby,W.R.; Smith,J.C.,Jr. Separation and identification of carotenoids and their oxidation products in the extracts of human plasma, *Anal.Chem.*, **1992**, *64*, 2111–2122.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum or plasma + 200 μL 25 μg/mL tocopheryl acetate in EtOH, vortex, add 400 μL butanol:ethyl acetate 50:50, mix for 1 min, add 20 mg sodium sulfate, vortex for 1 min, let stand at -20° for 20 min, centrifuge at 15000 g for 2 min, inject a 10 μL aliquot of the upper organic layer.

HPLC VARIABLES

Guard column: 5 μm C18
Column: 110 × 4.7 5 μm Partisphere 5 C18 (Whatman)
Mobile phase: MeOH:butanol:water 89.5:5:5.5
Column temperature: 45
Flow rate: 1.5
Injection volume: 10
Detector: UV 340 for 3 min, UV 290 for 1.5 min, UV 280 for 10.5 min, UV 450 for 7 min

CHROMATOGRAM

Retention time: 1.7
Internal standard: tocopheryl acetate (5.3)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: α-carotene, β-carotene, lycopene, δ-tocopherol, gamma-tocopherol, vitamin E, xanthophyll

KEY WORDS

serum; plasma; protect from light

REFERENCE

Lee,B.L.; Chua,S.C.; Ong,H.Y.; Ong,C.N. High-performance liquid chromatographic method for routine determination of vitamins A and E and β-carotene in plasma, *J.Chromatogr.*, **1992**, *581*, 41–47.

SAMPLE
Matrix: blood
Sample preparation: Dilute 1 mL serum 0.5-5 times with saline. Add 1 mL 0.25 μg/mL IS in EtOH to 1 mL diluted serum dropwise while vortexing, add 1.5 mL n-heptane, vortex for 1 min, centrifuge at 3000 rpm (Labofuge) for 15 min. Remove 1.3 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 40 μL MeCN:THF 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm ODS Hypersil
Mobile phase: MeCN:water:THF 81.3:5.7:13
Column temperature: 40
Flow rate: 0.4
Injection volume: 5
Detector: UV 326

CHROMATOGRAM
Retention time: 1.880
Internal standard: retinol acetate (2.216)
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: vitamin E (α-tocopherol), probucol, gamma-tocopherol, lycopene, α-carotene, β-carotene, metabolites

KEY WORDS
serum

REFERENCE
Schäfer Elinder,L.; Walldius,G. Simultaneous measurement of serum probucol and lipid-soluble antioxidants, *J.Lipid Res.*, **1992**, *33*, 131–137.

SAMPLE
Matrix: blood
Sample preparation: 20-500 μL Serum + 2 volumes EtOH + 1 mL ethyl acetate + 4-7 μL of a solution containing 16 mg/mL tocopheryl acetate, 2-3 μg/mL canthaxanthin, and 10 μg/mL retinoic acid, vortex for 30 s, centrifuge for 30 s, extract the pellet twice with 0.5-1 mL portions of ethyl acetate, extract the pellet with 0.5-1 mL hexane. Combine the supernatants, add 500 μL water, vortex, centrifuge. Remove the upper organic layer and evaporate it to dryness under a stream of argon, reconstitute the residue in 100 μL MeOH:dichloromethane 2:1, inject a 10-90 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Upchurch)
Column: 300 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:dichloromethane:MeOH:1-octanol 90:15:10:0.1
Flow rate: 1
Injection volume: 10-90
Detector: UV 325

CHROMATOGRAM
Retention time: 4
Internal standard: tocopheryl acetate, canthaxanthin, retinoic acid

OTHER SUBSTANCES
Extracted: beta carotene (UV 450), carotenoids (UV 450), vitamin E (UV 290)

KEY WORDS
protect from light; serum

REFERENCE

Barua,A.B.; Kostic,D.; Olson,J.A. New simplified procedures for the extraction and simultaneous high-performance liquid chromatographic analysis of retinol, tocopherols and carotenoids in human serum, *J.Chromatogr.*, **1993**, *617*, 257–264.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 100 μL retinyl hexanoate in MeOH, extract three times with 200 μL portions of hexane. Combine the hexane layers and evaporate them to dryness under a stream of argon, reconstitute the residue in 50 μL isopropanol:dichloromethane, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:dichloromethane:MeOH:n-butanol 90:15:10:0.1 containing 0.1% ammonium acetate
Flow rate: 1
Injection volume: 20
Detector: UV 300

CHROMATOGRAM

Internal standard: retinyl hexanoate

KEY WORDS

serum; comparison with capillary electrophoresis

REFERENCE

Ma,Y.; Wu,Z.; Furr,H.C.; Lammi-Keefe,C.; Craft,N.E. Fast minimicroassay of serum retinol (vitamin A) by capillary zone electrophoresis with laser-excited fluorescence detection, *J.Chromatogr.*, **1993**, *616*, 31–37.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum or plasma + 500 μL EtOH containing 4.27 μM retinyl acetate and 0.31 μM echinenone, rotamix for 30 s, add 2 mL n-hexane, rotamix for 30 s, centrifuge at 2000 g for 2 min, repeat extraction with 2 mL n-hexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μL THF, make up to 200 μL with EtOH, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 5 μm Spherisorb ODS1
Column: 250 × 4.6 5 μm Spherisorb ODS1
Mobile phase: Gradient. A was MeCN:MeOH 20:80 containing 100 mM ammonium acetate. B was 100 mM ammonium acetate in water. A:B from 90:10 to 100:0 over 12 min, maintain at 100:0 for 10 min, re-equilibrate at initial conditions for 5 min
Flow rate: 2
Injection volume: 50
Detector: UV 325 for 7.5 min, UV 292 for 5.5 min, then UV 450

CHROMATOGRAM

Retention time: 4.37
Internal standard: retinyl acetate (5.96), echinenone (15.15)
Limit of detection: 0.35 μM

OTHER SUBSTANCES

Extracted: β-carotene, cryptoxanthin, lutein, lycopene, vitamin E

KEY WORDS

plasma; protect from light; serum

REFERENCE

Zaman,Z.; Fielden,P.; Frost,P.G. Simultaneous determination of vitamins A and E and carotenoids in plasma by reversed-phase HPLC in elderly and younger subjects, *Clin.Chem.*, **1993**, *39*, 2229–2234.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma or serum + 200 μL 850 ng/mL retinyl acetate in EtOH, mix for 1 min, add 1 mL 0.4 g/L BHT (2,6-di-tert-butyl-4-methylphenol) in n-hexane, shake on a mechanical shaker for 10 min, centrifuge at 2000 g for 5 min, remove 800 μL of the supernatant, evaporate to dryness at 40° under a stream of nitrogen, reconstitute in 100 μL MeCN:THF:MeOH 68:22:7, inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Lichrosorb RP18
Column: 250 × 4.6 5 μm Nucleosil 100-5 C18
Mobile phase: MeCN:THF:MeOH 68:22:7 made up to 100 with 1% ammonium acetate
Flow rate: 1.5
Injection volume: 15
Detector: UV 325 for 3 min, UV 450 for 1.9 min, UV 290 for 2.5 min, UV 470 for 4.6 min, UV 450 for 3 min, then UV 325 for rest of run

CHROMATOGRAM
Retention time: 2.5
Internal standard: retinyl acetate (2.7)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: β-carotene, vitamin E (α-tocopherol), lutein, lycopene, α-carotene, zeaxanthin, trans β-carotene, δ-tocopherol

KEY WORDS
plasma; serum; protect from sunlight

REFERENCE
Bui,M.H. Simple determination of retinol, α-tocopherol and carotenoids (lutein, all-*trans*-lycopene, α- and β-carotenes) in human plasma by isocratic liquid chromatography, *J.Chromatogr.B*, **1994**, *654*, 129–133.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL IS in EtOH, vortex for 30 s, add 5 mL water, add 7.5 mL n-hexane, add 300 μL 2 M HCl, rotate for 10 min, centrifuge at 1250 g for 8 min. Remove the organic layer and evaporate it at room temperature under a stream of nitrogen. Dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb S5W
Mobile phase: n-Hexane:isopropanol:acetic acid 200:0.7:0.135
Flow rate: 0.9
Injection volume: 50
Detector: UV 350

CHROMATOGRAM
Retention time: 25
Internal standard: Ro 15-1570 (22)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: 13-cis-retinoic acid, trans-retinoic acid

KEY WORDS
plasma; normal phase

REFERENCE
Meyer,E.; Lambert,W.E.; De Leenheer,A.P. Simultaneous determination of endogenous retinoic acid isomers and retinol in human plasma by isocratic normal-phase HPLC with ultraviolet detection, *Clin.Chem.*, **1994**, *40*, 48–51.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL nonapreno-β-carotene and retinyl butyrate in EtOH, vortex for 10 s, add 1 mL hexane, vortex for 30 s, centrifuge at 1500 g for 5 min. Remove 900 μL of the hexane layer and evaporate it to a waxy or glassy consistency (not dryness) under vacuum, dissolve in 100 μL EtOH, add 100 μL MeCN, vortex, filter (0.45 μm), inject a 30 μL aliquot.

HPLC VARIABLES
Column: 1540 × 4.6 5 μm Ultramex C18 (Phenomenex)
Mobile phase: MeCN:EtOH 50:50 containing 0.1 mL/L diethylamine
Column temperature: 29
Flow rate: 0.9
Injection volume: 30
Detector: UV 300

CHROMATOGRAM
Retention time: 2.66
Internal standard: nonapreno-β-carotene (9.5, UV 450), retinyl butyrate (3.5, UV 300)
Limit of detection: 17 nM

OTHER SUBSTANCES
Extracted: β-carotene, vitamin E (α-tocopherol), lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, retinyl linoleate, retinyl oleate, retinyl palmitate, retinyl stearate

KEY WORDS
serum; use gold fluorescent lamps; hold sample at 4° before injection

REFERENCE
Sowell,A.L.; Huff,D.L.; Yeager,P.R.; Caudill,S.P.; Gunter,E.W. Retinol, α-tocopherol, lutein/zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, trans-β-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multiwavelength detection, *Clin.Chem.*, **1994**, *40*, 411–416.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 2 mL diluting agent (amber tube), mix for 10 s, add 5 mL n-hexane, vortex for 30 s, centrifuge at 1600 g at 4° for 5 min. Remove the organic phase and concentrate to < 1 mL under vacuum below 30°, evaporate the rest of the solvent under nitrogen, dissolve the residue in 25 μL MeCN:MeOH 2:1, mix for 30 s, centrifuge at 8000 g for 1 min, inject a 20 μL aliquot. (Diluting agent was MeCN:100 mM ammonium acetate 25:75, pH adjusted to 5.5 with acetic acid.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Chemcosorb 5-ODS-H
Mobile phase: MeCN:MeOH:100 mM ammonium acetate 46.7:23.3:30, pH adjusted to 7.0
Column temperature: 50
Flow rate: 1
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 31.9
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Simultaneous: 13-cis-retinoic acid, all-trans-retinoic acid

KEY WORDS
serum

REFERENCE

Takeda,N.; Yamamoto,A. Simultaneous determination of 13-cis- and all-trans-retinoic acids and retinol in human serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *657*, 53–59.

SAMPLE

Matrix: blood

Sample preparation: 10 μL Serum + 30 μL 200-500 ng/mL retinyl acetate in isopropanol:dichloroethane 2:1 + 5 μL glacial acetic acid, vortex for 30 s, centrifuge for 1 min, inject a 10-20 μL aliquot.

HPLC VARIABLES

Guard column: C18 (Upchurch)

Column: 150 × 4.6 Ultracarb 5 ODS30 (Phenomenex)

Mobile phase: MeCN:dichloromethane:MeOH 85:12:3 containing 0.1% ammonium acetate (dissolve ammonium acetate in MeOH first)

Flow rate: 1

Injection volume: 10-20

Detector: UV 335

CHROMATOGRAM

Retention time: 4.5

Internal standard: retinyl acetate (5.5)

Limit of detection: 10 ng/mL

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: retinoic acid

KEY WORDS

protect from light; serum

REFERENCE

Barua,A.B.; Kostic,D.; Barua,M.; Olson,J.A. Determination of retinol and retinoic acid in capillary blood by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 1459–1471.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 2.5 mL EtOH, mix for 5 min, add 5 mL n-hexane, mix vigorously, centrifuge at 2000 g for 5 min, repeat extraction with 3 mL n-hexane. Combine the n-hexane layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in dichloromethane, inject an aliquot.

HPLC VARIABLES

Guard column: μBondapak C18 Guard-Pak + 50 mm long C18 ODS(4) (Shimadzu)

Column: 250 × 4.6 5 μm Vydac 201 TP 54 C18

Mobile phase: MeOH:MeCN 90:10 (Every 100 injections wash column with MeOH:MeCN:dichloromethane 8:1:1.)

Flow rate: 1

Detector: UV 324

CHROMATOGRAM

Retention time: 3.8, 3.85 (9-cis)

OTHER SUBSTANCES

Extracted: beta carotene (UV 451), vitamin E (UV 291)

KEY WORDS

serum

REFERENCE

Ben-Amotz,A. Simultaneous profiling and identification of carotenoids, retinols, and tocopherols by high performance liquid chromatography equipped with three-dimensional photodiode array detection, *J.Liq.Chromatogr.*, **1995**, *18*, 2813–2825.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 100 μL retinyl acetate in MeOH, mix, extract three times with 200 μL portions of hexane. Combine the extracts and evaporate them to dryness under a stream of argon, reconstitute with 25 μL isopropanol:dichloromethane, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:water 95:5
Flow rate: 1
Injection volume: 20
Detector: UV 325

KEY WORDS

comparison with capillary electrophoresis; serum

REFERENCE

Shi,H.; Ma,Y.; Humphrey,J.N.; Craft,N.E. Determination of vitamin A in dried human blood spots by high-performance capillary electrophoresis with laser-excited fluorescence detection, *J.Chromatogr.B*, **1995**, *665*, 89–96.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 300 μL MeCN, vortex for 1.5 min, centrifuge at 10500 g for 1 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 10 × 4.5 5 μm CLC-G-ODS C18 (Shimadzu)
Column: 250 × 4.6 5 μm CLC-ODS C18 (Shimadzu)
Mobile phase: MeCN:water 90:10
Column temperature: 37
Flow rate: 2
Injection volume: 100
Detector: UV 328

CHROMATOGRAM

Retention time: 5.5
Limit of detection: 10 ng/mL

KEY WORDS

protect from light; serum

REFERENCE

Siddiqui,F.Q.; Malik,F.; Fazli,F.R. Determination of serum retinol by reversed-phase, *J.Chromatogr.B*, **1995**, *666*, 342–346.

SAMPLE

Matrix: blood

Sample preparation: 200 μL Serum + 200 μL 650 ng/mL tocopheryl acetate in MeOH, vortex for 30 s, add 200 μL n-hexane, shake for 15 min, centrifuge at 3000 rpm for 10 min. Remove 120 μL of the hexane layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 20 μL dichloromethane, add 100 μL MeCN:MeOH 50:50, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4 5 μm Nucleosil C18

Mobile phase: MeCN:MeOH:dichloromethane 50:45:5
Flow rate: 0.7
Injection volume: 50
Detector: F ex 325 em 480 for 5 min, then ex 295 em 330 for 10 min, then ex 325 em 480

CHROMATOGRAM
Retention time: 2.9
Internal standard: tocopheryl acetate (9.3)

OTHER SUBSTANCES
Extracted: vitamin E, γ-tocopherol, retinyl palmitate, β-carotene (UV 450), α-carotene (UV 450)

KEY WORDS
serum

REFERENCE
Yakushina,L.; Taranova,A. Rapid HPLC simultaneous determination of fat-soluble vitamins, including carotenoids, in human serum, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 715–718.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 100 μL Plasma + 100 μL 0.9% NaCl + 200 μL MeOH, vortex for 30 s, let stand for 10 min, add 400 μL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot. Tissue. Homogenize (liver with Mikro-dismembrator II in liquid nitrogen; other tissue with Ultra-Turrax) tissue with 3 mL 1% acetic acid containing 1 mg/mL ascorbic acid and 10 mM EDTA, add 2 mL MeOH, vortex for 30 s, let stand for 10 min, add 4 mL chloroform, vortex for 4 min. Remove the chloroform layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:dichloromethane:MeOH:water 70:10:15:5
Flow rate: 0.5 for 13 min, to 1 over 1 min, maintain at 1 for 10 min, to 1.5 over 1 min, maintain at 1.5 for 21 min, to 2 over 1 min, maintain at 2 for 10 min, return to 0.5 over 1 min, maintain at 0.5 for 2 min.
Injection volume: 50
Detector: UV 350

CHROMATOGRAM
Retention time: 6
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: beta carotene (UV 445), vitamin E (UV 292)

KEY WORDS
rat; protect from light; liver; plasma; lung

REFERENCE
Van Vliet,T.; Van Schaik,F.; Van Schoonhoven,J.; Schrijver,J. Determination of several retinoids, carotenoids and E vitamers by high-performance liquid chromatography. Application to plasma and tissues of rats fed a diet rich in either β-carotene or canthaxanthin, *J.Chromatogr.*, **1991**, *553*, 179–186.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, discard the effluent,

elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 3.5 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm Spherisorb RP-18
Mobile phase: MeCN:MeOH 70:30
Flow rate: 1.5
Injection volume: 10
Detector: UV 290

CHROMATOGRAM
Retention time: 2.6
Internal standard: anthraquinone (1.89)
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: vitamin E, vitamin E acetate

KEY WORDS
plasma; SPE

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE
Matrix: cheese
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70° for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10. Combine the organic layers and evaporate them to dryness, dissolve the residue in 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si
Mobile phase: Gradient. A was n-hexane:isopropanol 99:1. B was n-hexane. A:B 50:50 for 7 min; to 90:10 over 4 min, maintain at 90:10 for 7 min, to 50:50 over 1 min, maintain at 50:50 for 4 min. (About every 100 injections recondition column with 50 mL dichloromethane, 50 mL isopropanol, and 50 mL dichloromethane.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 325 em 475 (vitamin A), UV 450 (β-carotene), and F ex 325 em 475 for 3.5 min, ex 280 em 475 for 10.5 min, ex 325 em 475 for 9 min (others)

CHROMATOGRAM
Retention time: 18
Limit of detection: 0.32 ng

OTHER SUBSTANCES
Extracted: β-carotene, vitamin E (α-tocopherol), β-tocopherol, gamma-tocopherol, δ-tocopherol, 13-cis-retinol

KEY WORDS
normal phase; cheese

REFERENCE
Panfili,G.; Manzi,P.; Pizzoferrato,L. High-performance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses, *Analyst*, **1994**, *119*, 1161–1165.

SAMPLE
Matrix: culture media
Sample preparation: 100 μL Culture media + 200 μL ice-cold EtOH, mix thoroughly, let stand for 15 min, centrifuge at 12000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: Whatman CO:PELL ODS guard column
Column: 100 × 8 5 μm Nova-Pak C18 (radial-packed)
Mobile phase: MeOH:100 mM pH 7.0 ammonium acetate 90:10
Flow rate: 1
Detector: UV 340

CHROMATOGRAM
Retention time: 12.50

OTHER SUBSTANCES
Extracted: isotretin, motretinid, acitretin, all-trans-retinoic acid, retinal, etretinate

REFERENCE
Kochhar,D.M.; Penner,J.D.; Minutella,L.M. Biotransformation of etretinate and developmental toxicity of etretin and other aromatic retinoids in teratogenesis bioassays, *Drug Metab.Dispos.*, **1989**, *17*, 618–624.

SAMPLE
Matrix: flour, milk
Sample preparation: Mix 5 g powdered milk or flour with 20 mL 500 g/L NaOH in water. Warm at 30° for 3 min, add 100 mL EtOH and 2 mL 200 g/L hydroquinone in EtOH. Heat at 80° for 30 min. Cool, add 100 mL water and 50 mL diethyl ether. Shake and add 50 mL petroleum ether, shake. Remove the organic layer and reextract the aqueous layer as above. Combine the organic layers, filter (cellulose), wash twice with 100 mL water, evaporate to dryness under reduced pressure, dissolve in 1 mL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Zorbax ODS
Column: 150 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 92:8
Column temperature: 25 ± 1
Flow rate: 1.5
Injection volume: 20
Detector: UV 330

CHROMATOGRAM
Retention time: 13.45
Limit of detection: 20 ng/g
Limit of quantitation: 120 ng/g

KEY WORDS
powdered milk

REFERENCE
Ake,M.; Mandrou,B.; Malan,A. Determination of vitamin A in milk and flour consumed by one- to four-year-old children in Côte d'Ivoire, *J.AOAC Int.*, **1998**, *81*, 111–114.

SAMPLE
Matrix: food
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70, for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10 (Analyst 1994, 119, 1161). Combine the organic layers and evaporate them to dryness, dissolve the residue in MeOH:dichloromethane 90:10, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Supelco C18
Mobile phase: MeOH
Flow rate: 2
Detector: F ex 325 em 475

CHROMATOGRAM
Retention time: 2.4
Limit of detection: 400 pg

OTHER SUBSTANCES
Extracted: vitamin E (α-tocopherol, β and γ-tocopherol, δ-tocopherol) (F ex 280 em 325), sterols (UV 208), carotenes (UV 450)

KEY WORDS
cheese

REFERENCE
Manzi,P.; Panfili,G.; Pizzoferrato,L. Normal and reversed-phase HPLC for more complete evaluation of tocopherols, retinols, carotenes and sterols in dairy products, *Chromatographia*, **1996**, *43*, 89–91.

SAMPLE
Matrix: food
Sample preparation: 500 mg Cheese + 2 mL 60% KOH + 2 mL 95% EtOH + 1 mL 1% NaCl + 5 mL 6% pyrogallol in EtOH, flush tube with nitrogen, seal, heat at 70, for 30 min, cool in ice water, add 15 mL 1% NaCl, extract twice with 15 mL portions of n-hexane:ethyl acetate 90:10 (Analyst 1994, 119, 1161). Combine the organic layers and evaporate them to dryness, dissolve the residue in n-hexane:2-propanol 99:1, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Si (Beckman)
Mobile phase: Gradient. A was n-hexane:isopropanol 99:1. B was n-hexane. A:B 50:50 for 7 min; to 90:10 over 4 min, maintain at 90:10 for 7 min, to 50:50 over 1 min, maintain at 50:50 for 4 min. (About every 100 injections recondition column with 50 mL dichloromethane, 50 mL isopropanol, and 50 mL dichloromethane.)
Flow rate: 1.5
Detector: F ex 325 em 475

CHROMATOGRAM
Retention time: 15 (13-cis-retinol), 17.3 (all trans-retinols)
Limit of detection: 100 pg (13-cis-retinol), 300 pg (all trans-retinols)

OTHER SUBSTANCES
Extracted: vitamin E (α, β, γ, δ-tocopherol) (F ex 280 em 325), sterols (UV 208), carotenes (UV 450)

KEY WORDS
cheese; normal phase

REFERENCE
Manzi,P.; Panfili,G.; Pizzoferrato,L. Normal and reversed-phase HPLC for more complete evaluation of tocopherols, retinols, carotenes and sterols in dairy products, *Chromatographia*, **1996**, *43*, 89–91.

SAMPLE
Matrix: food, milk, tissue
Sample preparation: 40 mL Fluid sample or 1-10 g solid sample homogenized with 30 mL water + 12 mL 60% KOH + 80 mL EtOH + 0.5 mL 1% BHT in EtOH + 0.5 g ascorbic acid, stir for 16 h under nitrogen, make up to 250 mL with water and EtOH so that the water:EtOH ratio is 50:50, add 20 mL to a 150 × 25 Kieselguhr SPE cartridge, let stand for 20 min, elute with 50 mL light petroleum, force out all the eluate with nitrogen, evaporate the eluate under reduced pressure, take up the residue in 2-50 mL isooctane, inject an aliquot.

HPLC VARIABLES
Guard column: 7 × 2 3 μm Spherisorb SW silica gel
Column: 100 × 2 3 μm Spherisorb SW silica gel
Mobile phase: n-Hexane:1-octanol 99.7:0.3
Flow rate: 0.4
Injection volume: 10
Detector: UV 325

CHROMATOGRAM
Retention time: 6.4 (11-cis), 7.1 (11,13-di-cis), 8.4 (13-cis), 9.3 (9,13-di-cis), 11.0 (9-cis), 11.6 (7-cis), 12.5 (all-trans)
Limit of detection: 140 ng/mL

KEY WORDS
normal phase; liver; sausage; sour cream; food; SPE

REFERENCE
Brinkmann,E.; Dehne,L.; Oei,H.B.; Tiebach,R.; Baltes,W. Separation of geometrical retinol isomers in food samples by using narrow-bore high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *693*, 271–279.

SAMPLE
Matrix: formula, reference material
Sample preparation: 10 g Zero-control reference material (ZRM) powder + 50 g hot water, mix. 6.5 g Reconstituted ZRM or 3.5 g concentrated commercial formula + 10 mL boiling isopropanol, mix, add 7.5 g anhydrous magnesium sulfate to the ZRM and 4 g to the concentrated commercial formula, mix thoroughly, add 25 mL hexane:ethyl acetate 85:15, add 1 mL 360 .micro g/mL BHT, mix, homogenize (Polytron) for 1 min, filter through 60 mL coarse-porosity fritted glass filter using vacuum, wash with two 15 mL portions of hexane:ethyl acetate 85:15. Re-extract with 20 mL hexane:ethyl acetate 85:15 and 5 mL isopropyl alcohol, homogenize for 1 min, filter through 60 mL coarse-porosity fritted glass filter using vacuum, wash with two 15 mL portions of hexane:ethyl acetate 85:15. Mix the combined filtrate with 500 mg anhydrous magnesium sulfate, evaporate to dryness, add 15 mL hexane to the residue, filter (0.45 μm nylon) using vacuum, wash with three 7 mL portions of hexane, evaporate to 1 mL with nitrogen at 45°, dilute to 10 mL with hexane, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosorb Si 60
Mobile phase: Hexane:isopropanol 99.875:0.125
Flow rate: 1
Injection volume: 50
Detector: F ex 325 em 470

CHROMATOGRAM
Retention time: 3.4 (vitamin A palmitate)
Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES
Also analyzed: vitamin E

KEY WORDS
normal phase; soy-based infant formula

REFERENCE
Chase,G.W.,Jr.; Long,A.R.; Eitenmiller,R.R. Liquid chromatographic method for analysis of all-rac-α-tocopheryl acetate and retinyl palmitate in soy-based infant formula using a zero-control reference material (ZRM) as a method development tool, *J.AOAC Int.*, **1998**, *81*, 577–581.

SAMPLE
Matrix: formulations

Sample preparation: Reconstitute 28 g milk based infant formula with ca. 145 g 78-80° water, mix thoroughly. Add 15 mL boiling isopropanol to 6.5 g reconstituted infant formula, mix thoroughly. Add 7.5 g magnesium sulfate, 30 mL hexane:ethyl acetate 85:15 and 1 mL BHT. Homogenize mixture for 1 min, filter through a coarse porosity glass filter by vacuum, wash the magnesium sulfate cake with two 15 mL portions of hexane:ethyl acetate 85:15. Repeat the extraction with 5 mL isopropanol and 15 mL hexane:ethyl acetate 85:15. Add 1 g magnesium sulfate to the combined filtrate and evaporate it to dryness under nitrogen. Dissolve the residue in 10 mL hexane, filter (0.45 μm), evaporate to a volume of less than 5 mL at 45°. Dilute to 10 mL with mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosorb Si 60
Mobile phase: Hexane:isopropanol 99.5:0.5
Flow rate: 0.5
Injection volume: 50
Detector: F ex 325 em 470

CHROMATOGRAM

Retention time: 2.9
Limit of detection: 187 ng/mL

KEY WORDS

infant formula; normal phase

REFERENCE

Chase,Jr.,G.W.; Eitenmiller,R.R.; Long,A.R. Liquid chromatographic analysis of all-RAC-α-tocopheryl acetate, tocopherols, and retinyl palmitate in SRM 1846, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3317–3327.

SAMPLE

Matrix: formulations
Sample preparation: Tablets, capsules. Add 5 crushed tablets or the contents from 5 capsules to 10 mL DMSO, add 15 mL hexane, shake at 60° for 45 min, centrifuge at 3000 rpm for 10 min, remove hexane layer, add 15 mL hexane, vortex for 5 min at room temperature, remove hexane layer, repeat hexane extraction three more times, combine all hexane layers, filter, make up to 100 mL with hexane, dilute if necessary, inject a 100 μL aliquot. Syrup. 10 mL Syrup + 10 mL DMSO + 15 mL ether:hexane 10:90, vortex for 5 min, remove hexane layer, repeat extraction four more times, combine the extracts, evaporate with nitrogen until the ether is removed, filter, make up to 100 mL with hexane, dilute if necessary, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm amino bonded phase (Chromatography Sciences Co.)
Mobile phase: Hexane:isopropanol 99:1
Flow rate: 1-2
Injection volume: 100
Detector: UV 265

CHROMATOGRAM

Retention time: 12 (13-cis), 13 (9-cis), 14.5 (all-trans)

OTHER SUBSTANCES

Simultaneous: cholecalciferol (vitamin D3), ergocalciferol (vitamin D2)

KEY WORDS

work under subdued light; tablets; capsules; syrup

REFERENCE

Beaulieu,N.; Curran,N.M.; Gagné,C.; Gravelle,M.; Lovering,E.G. Liquid chromatographic methods for vitamins A and D in multivitamin-mineral formulations, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 247–254.

SAMPLE

Matrix: formulations

Sample preparation: Weigh out a sample containing about 2500 UI of vitamin A, add 20 mL MeOH, protect from light, stir vigorously for 2-2.5 h, filter (0.45 μm), inject a 2 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pack C18
Mobile phase: MeCN:THF:water 55:37:8
Flow rate: 1.5
Injection volume: 2
Detector: UV 325

CHROMATOGRAM
Retention time: 1.08 (retinol), 1.25 (retinyl acetate)

KEY WORDS
protect from light; capsules; tablets

REFERENCE
Genestar,C.; Grases,F. Determination of vitamin A in pharmaceutical preparations by high-performance liquid chromatography with diode-array detection, *Chromatographia*, **1995**, *40*, 143–146.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a fine powder, weigh out 100-150 mg, mix with sea sand. Extract with supercritical carbon dioxide (Dionex) in the dynamic mode at 250 atmospheres and 40° for 15 min (restrictor 60°, gaseous flow rate 190-220 mL/min), collect in 6 mL THF at 0°, make up to 10 mL with THF, inject an aliquot. [Alternatively, add 5 crushed tablets or the contents from 5 capsules to 10 mL DMSO, add 15 mL hexane, shake at 60° for 45 min, centrifuge at 3000 rpm for 10 min, remove hexane layer, add 15 mL hexane, vortex for 5 min at room temperature, remove hexane layer, repeat hexane extraction three more times, combine all hexane layers, dilute with THF, inject a 25 μL aliquot. J.Assoc.Off.Anal.Chem. 1989, 72, 247.]

HPLC VARIABLES
Guard column: 4 × 4 5 μm (Merck)
Column: 250 × 4 5 μm Lichrospher CH-8
Mobile phase: MeOH:MeCN 75:25
Flow rate: 1.3
Injection volume: 50
Detector: UV 325

CHROMATOGRAM
Retention time: 9 (vitamin A palmitate)

OTHER SUBSTANCES
Simultaneous: vitamin E acetate (UV 280)

KEY WORDS
tablets; SFE

REFERENCE
Scalia,S.; Ruberto,G.; Bonina,F. Determination of vitamin A, vitamin E, and their esters in, *J.Pharm.Sci.*, **1995**, *84*, 433–436.

SAMPLE
Matrix: formulations
Sample preparation: Mix 100-150 mg of the formulation with celite and extract with supercritical carbon dioxide at 250 atmospheres at 40° at 190-220 mL/min with the restrictor at 100° (Dionex SFE-703), collect in 4 mL THF:MeOH 80:20 at 0°, make up to 5 mL, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-Pak (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH 25:75
Flow rate: 1.5
Detector: UV 325

CHROMATOGRAM
Retention time: 10 (vitamin A palmitate)

OTHER SUBSTANCES
Simultaneous: vitamin E acetate (UV 280)

KEY WORDS
SFE; cream; lotion; protect from light

REFERENCE
Scalia,S.; Renda,A.; Ruberto,G.; Bonina,F.; Menegatti,E. Assay of vitamin A palmitate and vitamin E acetate in cosmetic creams and lotions by supercritical fluid extraction and HPLC, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 273–277.

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20 min. Extract twice with 10 mL n-hexane. Evaporate the extracts to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5μm C18 (Alltech)
Mobile phase: MeOH:EtOH 80:20 (A) or EtOH:water 95:5 (B)
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM
Retention time: 4.45 (A), 4 (B)

OTHER SUBSTANCES
Extracted: retinal, isotretinoin, tretinoin, vitamin D2, vitamin D3, vitamin E, vitamin K1, vitamin K2

REFERENCE
Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE
Matrix: milk
Sample preparation: Add 500 mg ascorbic acid, 50 mL EtOH, and 10 mL 60% KOH to 25 g liquid or reconstituted powdered milk under a nitrogen stream, stir overnight at room temperature. Extract with three 50 mL portions of n-hexane and two 25 mL portions of n-hexane by shaking for 2 min each. Combine the n-hexane extracts, wash with 50 mL portions of water containing a few drops of phenolphthalein until the aqueous phase appears colorless, add 1 g butylated hydroxytoluene, filter through a Whatman No.1 filter containing 20 g anhydrous sodium sulfate, concentrate the filtrate under reduced pressure at 40°, reconstitute with 10 mL MeOH, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: Tracer Spherisorb ODS 2 C18
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeCN:MeOH:water 1:95:4
Injection volume: 20

Detector: UV 323 for 14 min then UV 292

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: vitamin E

REFERENCE
Albal-Hurtado,S.; Novella-Rodríguez,S.; Veciana-Nogués,M.; Mariné-Font,A. Determination of vitamins A and E in infant milk formulae by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 243–246.

SAMPLE
Matrix: milk
Sample preparation: 1 g Powdered milk or 25 mL liquid milk + alcoholic KOH, let stand overnight, extract with hexane. Remove the organic layer and evaporate it to dryness, reconstitute the residue in MeOH, filter, inject an aliquot. (Alcoholic KOH was prepared from 50 mL EtOH and 15 mL 60% KOH in water.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid/sodium acetate
Flow rate: 1.25
Injection volume: 10
Detector: E, EG & G PAR Model 400, MP 1304 glassy carbon series dual electrode, E1 (upstream) -1100 mV, E2 (downstream) +700 mV (Condition electrodes for 30 min at E1 -1200 mV and E2 +1500 mV at the start of each day.)

CHROMATOGRAM
Retention time: 4
Limit of detection: 0.06 ng

OTHER SUBSTANCES
Extracted: vitamin E

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez Perez,A.; Gomez Perez,M.C.; Fernandez Moro,M.A.; Hernandez Mendez,J. Determination of vitamins A, E and K1 in milk by high-performance liquid chromatography with dual amperometric detection, *Analyst*, **1995**, *120*, 2489–2492.

SAMPLE
Matrix: milk
Sample preparation: Dilute milk to 30% with water, mix with reagent and pass through a 5 m × 0.5 mm i.d. PTFE tube knotted reactor at 1.25 mL/min, mix with 2.5 M acetic acid, pass onto a Sep-Pak Plus C18 SPE cartridge for 5 min, wash SPE cartridge with MeOH:water 40:60 for 4 min, elute SPE cartridge with MeOH for 4 min, inject the last 100 μL of the eluate. (Reagent was 50 mL EtOH + 15 mL 60% aqueous NaOH + 5 mL 10% ascorbic acid.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP18 (Brownlee)
Column: 220 × 4.6 5 μm OD-224 RP18 (Brownlee)
Mobile phase: MeOH:water 99:1 containing 2.5 mM acetic acid-sodium acetate
Flow rate: 1
Injection volume: 100
Detector: E, glassy carbon working electrode +1300 mV

CHROMATOGRAM
Retention time: 6

Limit of detection: 34.9 nM

OTHER SUBSTANCES
Extracted: vitamin E, vitamin D3

KEY WORDS
SPE; derivatization

REFERENCE
Delgado-Zamarreño,M.M.; Sanchez-Perez,A.; Gomez-Perez,M.C.; Hernandez-Mendez,J. Directly coupled sample treatment-high-performance liquid chromatography for on-line automatic determination of liposoluble vitamins in milk, *J.Chromatogr.A*, **1995**, *694*, 399–406.

SAMPLE
Matrix: silicone oils
Sample preparation: Condition a 1 g Si Bond-Elut SPE cartridge with 5 mL n-hexane. Mix 1 g silicone oil with 2 mL dichloromethane, vortex for 2 min, centrifuge at 3000 g. Withdrawn the supernatant, repeat this procedure twice, filter (0.45 μm), heat the filtrate at 50°, expose to a stream of helium for 30 min. Add 2.5 μg retinol acetate, 2.5 μg α-tocopherol acetate, and 25 μg BHT. Add the mixture to the SPE cartridge, elute with 500 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax C8
Mobile phase: Gradient. A was MeCN:200 mM ammonium acetate 72:25. B was MeOH:water 95:5. A:B 100:0 for 10 min, to 0:100 over 1 min, maintain at 0:100 for 14 min
Flow rate: 2 for 10 min then 1.5
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 4
Internal standard: retinol acetate (9.5)
Limit of detection: 51.4 ng/mL
Limit of quantitation: 171.2 ng/mL

OTHER SUBSTANCES
Extracted: cholesterol (UV 210), retinal (UV 350), retinoic acid (UV 350), α-tocopherol acetate, vitamin E (UV 210)

KEY WORDS
ophthalmic silicone oils; SPE

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Marinero,P. Simultaneous HPLC determination of cholesterol, α-tocopherol, retinol, retinal and retinoic acid in silicone oils used as vitreous substitutes in eye surgery, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1151–1167.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 8 μm Unisphere-PBD (polybutadiene on alumina) (Biotage, Charlottesville, VA)
Mobile phase: MeOH:water 92:8
Flow rate: 1
Detector: UV 330

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: retinoic acid, retinol acetate, vitamin E

REFERENCE
Jedrejewski,P.T.; Taylor,L.T. Comparison of silica-, alumina-, and polymer-based stationary phases for reversed-phase liquid chromatography, *J.Chromatogr.Sci.*, **1995**, *33*, 438–445.

SAMPLE
Matrix: tissue
Sample preparation: 50 mg Tissue + 50 μL 560 μg/mL vitamin K in EtOH, extract twice with 1 mL n-hexane using a sonicator, centrifuge at 2000 g for 5 min. Evaporate the supernatant to dryness, reconstitute it in 200 μL chloroform:MeOH 25:75, inject an aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil 120 C18
Mobile phase: MeOH:water 96.5:3.5
Column temperature: 40
Flow rate: 2
Detector: UV 325

CHROMATOGRAM
Retention time: ca. 2
Internal standard: vitamin K (9)

OTHER SUBSTANCES
Extracted: vitamin A_p, vitamin E (F ex 295 em 350)

KEY WORDS
rat; liver; placenta; brain

REFERENCE
Barbas,C.; Castro,M.; Bonet,B.; Viana,M.; Herrera,E. Simultaneous determination of vitamins A and E in rat tissues by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 415–420.

SAMPLE
Matrix: tissue
Sample preparation: 100-120 mg Tadpole embryos + 100 mL isopropanol, sonicate on ice, vortex for 1 min, centrifuge at 4000 g at 4° for 20 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 4 10 μm LiChrosorb RB 18
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: Gradient. MeOH:40 mM pH 7.3 ammonium acetate from 55:45 to 100:0 over 20 min
Flow rate: 1.6
Detector: UV 354

CHROMATOGRAM
Retention time: 15.2

OTHER SUBSTANCES
Extracted: isotretinoin, tretinoin, metabolites

KEY WORDS
handle under yellow light; tadpoles; embryos

REFERENCE
Creech Kraft,J.; Kimelman,D.; Juchau,M.R. *Xenopus Laevis*: A model system for the study of embryonic retinoid metabolism. I. Embryonic metabolism of 9-*cis*- and all-*trans*-retinals and retinols and their corresponding acid forms, *Drug Metab.Dispos.*, **1995**, *23*, 72–82.

SAMPLE
Matrix: tissue
Sample preparation: 100-120 mg Frog embryos + 100 μL isopropanol, sonicate on ice, vortex for 1 min, centrifuge at 4° at 4000 g for 20 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 4 10 μm LiChrosorb RB 18
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: Gradient. MeOH:40 mM pH 7.3 ammonium acetate from 55:45 to 100:0 over 18 min.
Flow rate: 1.6
Detector: UV 354

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Extracted: retinoic acid

KEY WORDS
frog; embryo

REFERENCE
Creech Kraft,J.; Juchau,M.R. *Xenopus laevis*: A model system for the study of embryonic retinoid metabolism. III. Isomerization and metabolism of all-*trans*-retinoic acid and 9-*cis*-retinoic acid and their dysmorphogenic effects in embryos during neurulation, *Drug Metab.Dispos.*, **1995**, *23*, 1058–1071.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (7 mL Tenbroeck grinder, Bioblock) 2-10 mg liver and 1.5 mL 1% pyrogallol in EtOH, shake at 4° for 30 min, add 100 μL water, add 3 mL n-hexane, extract, centrifuge at 4° at 3000 g for 15 min, repeat extraction. Evaporate the hexane layers separately as rapidly as possible under a stream of nitrogen at 50°, reconstitute with 200 μL 30 μM retinyl acetate in MeOH, inject a 50 μL aliquot. (For total retinol homogenize liver with 1.5 mL 10% KOH in 95% EtOH, heat at 60° for 30 min, cool in an ice bath, add 800 μL water, extract twice with 1.9 mL n-hexane. Evaporate the hexane layers separately as rapidly as possible under a stream of nitrogen at 50°, reconstitute with 200 μL MeOH, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-8 (Brownlee)
Column: 250 × 4.6 5 μm Supelcosil LC-8
Mobile phase: MeOH:water 94:6
Flow rate: 1.5
Injection volume: 50
Detector: UV 325

CHROMATOGRAM
Retention time: 3
Internal standard: retinyl acetate (3.7)
Limit of quantitation: 6 nmole/g

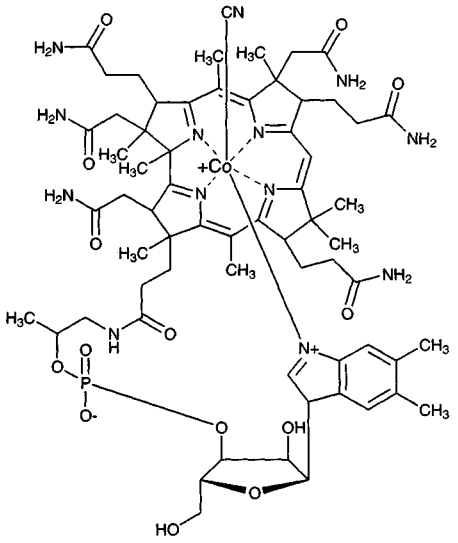
OTHER SUBSTANCES
Extracted: retinyl oleate, retinyl palmitate, retinyl stearate

KEY WORDS
liver; protect from light

REFERENCE
Got,L.; Gousson,T.; Delacoux,E. Simultaneous determination of retinyl esters and retinol in human livers by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *668*, 233–239.

Vitamin B12

Molecular formula: $C_{63}H_{88}CoN_{14}O_{14}P$
Molecular weight: 1355.38
CAS Registry No.: 68-19-9
Merck Index: 10152



SAMPLE

Matrix: blood, feces

Sample preparation: Feces. Condition a Sep-Pak C18 SPE cartridge with 2 mL MeCN and 6 mL water. 1 g Feces + 3 mL buffer, rotate at 4° for 24 h, centrifuge at 19000 g for 1 h. Remove 3 mL of the supernatant and add cadmium acetate to a concentration of 200 mM, let stand for 2 h, add four volumes of EtOH preheated to 80°, mix vigorously, heat at 80° for 20 min, cool in an ice bath, centrifuge at 2000 g for 10 min, remove the supernatant, mix the precipitate with two volumes of cold EtOH:water 80:20, centrifuge. Combine the supernatants and evaporate them to dryness under reduced pressure at 40°, reconstitute the residue in 2 mL water, add to the SPE cartridge, wash with 12 mL water, elute with 6 mL t-butanol:water 20:80, evaporate the eluate to dryness, reconstitute with 2 mL 1% acetic acid, add to the Amberlite XAD2 column, wash with 12 mL 1% acetic acid, wash with 12 mL MeOH:1% acetic acid 10:90, elute with 30 mL MeOH:1% acetic acid 50:50, lyophilize the eluate at -80°, reconstitute with 2 mL water, filter (0.2 μm), lyophilize, reconstitute with 300 μL 1% acetic acid, inject a 250 μL aliquot. Plasma. Condition a Sep-Pak C18 SPE cartridge with 2 mL MeCN and 6 mL water. Add cadmium acetate to a concentration of 200 mM, let stand for 2 h, add four volumes of EtOH preheated to 80°, mix vigorously, heat at 80° for 20 min, cool in an ice bath, centrifuge at 2000 g for 10 min, remove the supernatant, mix the precipitate with two volumes of cold EtOH:water 80:20, centrifuge. Combine the supernatants and evaporate them to dryness under reduced pressure at 40°, reconstitute the residue in 2 mL water, add to the SPE cartridge, wash with 12 mL water, elute with 6 mL t-butanol:water 20:80, evaporate the eluate to dryness, lyophilize the eluate at -80°, reconstitute with 2 mL water, filter (0.2 μm), lyophilize, reconstitute with 300 μL 1% acetic acid, inject a 250 μL aliquot (Meth. Enzymol. 1986, 123, 3). (Buffer was 100 mM pH 7.4 sodium phosphate buffer containing 5 U/mL aprotinin, 0.02 mM phenylmethylsulfonyl fluoride, 3 mM sodium azide, and 0.05% Triton X100. Prepare Amberlite XAD2 column as follows. Suspend 30 g resin in 50 mL acetone, filter, wash with 50 mL acetone, dry at 80°, suspend in 200 mL MeOH, allow to settle, discard the supernatant, repeat 2-4 times until supernatant is clear, suspend in 100 mL MeOH, fill a 330 × 8 glass column to a bed height of 40 mm (Meth. Enzymol. 1986, 123, 3).)

HPLC VARIABLES

Column: 250 × 5 5 μm LiChrospher RP18 glass column
Mobile phase: Gradient. A was 85 mM phosphoric acid adjusted to pH 3.0 with triethanolamine. B was MeCN. A:B from 90:10 to 50:50 over 20 min.
Flow rate: 0.5
Injection volume: 250
Detector: UV 365

CHROMATOGRAM

Retention time: 17.5

KEY WORDS
protect from light; plasma; SPE

REFERENCE
Djalali,M.; Gueant,J.-L.; Lambert,D.; el Kholty,S.; Saunier,M.; Nicolas,J.-P. High-performance liquid chromatographic separation and dual competitive binding assay of corrinoids in biological material, *J.Chromatogr.*, **1990**, *529*, 81–91.

SAMPLE
Matrix: blood, formulations, urine
Sample preparation: Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma, urine. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-18
Mobile phase: Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 17.12
Internal standard: xanthine (4.65)
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: ascorbic acid, folic acid, niacin, niacinamide, riboflavin

KEY WORDS
plasma; SPE; tablets; injections

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM
Retention time: 3.777

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, formulations, premix
Sample preparation: Weigh out amount containing 20-100 μg vitamin B12, extract with DMSO: water 50:50 (or 0.5% ammonium pyrrolidine dithiocarbamate and 2% citric acid in DMSO: water 50:50 for samples containing ascorbic acid with iron or copper) by shaking at 55° for 45 min, centrifuge, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 2540 × 4.6 μBondapak C18
Mobile phase: Gradient. MeOH:water from 15:85 to 50:50 over 15 min.
Detector: UV 546

CHROMATOGRAM
Retention time: 8
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: riboflavin

REFERENCE
Hudson,T.S.; Subramanian,S.; Allen,R.J. Determination of pantothenic acid, biotin, and vitamin B12 in nutritional products, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 994–998.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

CHROMATOGRAM
Retention time: 22
Limit of quantitation: ≤300 ng/mL

OTHER SUBSTANCES
Extracted: thiamine, riboflavin, pyridoxine, folic acid, niacinamide, pyridoxal, pyridoxamine

REFERENCE
Albalá-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 6 5 μm Capcell Pak C8 (Shiseido, Japan)
Mobile phase: MeOH:50 mM KH_2PO_4 containing 5 mM tetra-n-butylammonium phosphate 15:85, adjusted to pH 2.6 with 5% orthophosphoric acid (After one week of use, wash the column with water and MeOH:water 70:30 at 1 mL/min for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 10-20
Detector: UV 215

CHROMATOGRAM
Retention time: 27

OTHER SUBSTANCES
Simultaneous: chlorpheniramine, dipotassium glycyrrizate, fumaric acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, maleic acid, neostigmine methylsulfate, pyridoxine, tetrahydrozoline
Noninterfering: chondroitin sulfate, lysozyme

KEY WORDS
ophthalmic solutions; ion-pair agents

REFERENCE
Yamato,S.; Nakajima,M.; Shimada,K. Simultaneous determination of chlorpheniramine and maleate by high-performance liquid chromatography using tetra-n-butylammonium phosphate as an ion-pair reagent, *J.Chromatogr.A*, **1996**, *731*, 346–350.

SAMPLE
Matrix: formulations
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeCN and 10 mL water. Dissolve 20 g powder formulation in 60 mL water, add 10 g NaCl, heat at 50° until completely dissolved, let stand at room temperature for 30 min, make up to 100 mL with water, wash with 10 mL hexane for 3 min. Add the aqueous layer to the SPE cartridge, elute with 8 mL MeCN:water 50:50, evaporate the eluate to dryness under reduced pressure at 50°, reconstitute with 4 mL water, inject a 2 mL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Capcellpak C18 (Shiseido)
Mobile phase: MeCN:water 13:87
Flow rate: 0.6
Injection volume: 2000
Detector: UV 550

CHROMATOGRAM
Retention time: 13
Limit of quantitation: 2.2 ng/g

KEY WORDS
SPE; powders

REFERENCE
Iwase,H. Ultramicrodetermination of cyanocobalamin in elemental diet by solid-phase extraction and high-performance liquid chromatography with visible detection, *J.Chromatogr.*, **1992**, *590*, 359–363.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 9.5

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, pyridoxine, riboflavin, saccharin, thiamine, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE
Matrix: solutions

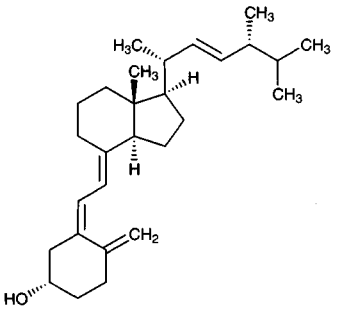
HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 1.9

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxal, pyridoxamine, thiamine, riboflavin, pyridoxine

REFERENCE
J & W Catalog, 1992-3, p. 277.

Vitamin D2



Molecular formula: $C_{28}H_{44}O$
Molecular weight: 396.66
CAS Registry No.: 50-14-6
Merck Index: 10156

SAMPLE

Matrix: blood

Sample preparation: 3-5 mL Plasma + 3 volumes ether, shake horizontally at 120 oscillations/min for 5 min, let stand for 1-2 min, freeze in dry ice/acetone, repeat ether extraction, extract aqueous layer with 4 volumes dichloromethane:MeOH 75:25, shake for 3-5 min, add 1 mL MeOH, shake for 15 s. Remove the organic layer and wash it twice with 100 mM pH 10.5 phosphate buffer, combine the dichloromethane and ether extracts and evaporate them to dryness under a stream of nitrogen, chromatograph on a 155 × 6 Sephadex LH-20 column with hexane:chloroform:MeOH 90:10:10, discard first 1 mL eluate, collect next 2.5 mL eluate. Evaporate to dryness under a stream of nitrogen, reconstitute with 500 µL hexane:chloroform 95:5 and chromatograph on a 145 × 6 Lipidex 5000 column (Packard) with hexane:chloroform 95:5, discard first 6 mL, collect next 4 mL. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 150 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.5 Zorbax Sil
Mobile phase: Dichloromethane:isopropanol 99.75:0.25
Flow rate: 2
Injection volume: 150
Detector: UV 254

CHROMATOGRAM

Retention time: 16

OTHER SUBSTANCES

Interfering: vitamin D3

KEY WORDS

plasma; normal phase; turkey; chicken; cow; pig; sheep; SPE

REFERENCE

Horst,R.L.; Littledike,E.T.; Riley,J.L.; Napoli,J.L. Quantitation of vitamin D and its metabolites and their plasma concentrations in five species of animals, *Anal.Biochem.*, **1981**, *116*, 189–203.

SAMPLE

Matrix: blood

Sample preparation: 2-3 mL Serum + 3.75 volumes hexane:isopropanol 1:2, shake for 30 min, let stand for 5 min, add 1.25 volume hexane, shake for 5 min, centrifuge at 600 g for 5 min, remove the upper organic layer, extract the aqueous layer twice with 1.25 volumes of hexane. Combine all the organic layers and evaporate them to dryness under a stream of nitrogen at 35°, reconstitute the residue in 50 µL hexane:isopropanol 80:20 and inject on to a 200 × 1 column of Kieselgel Si-60, Size A (Merck) and elute with hexane:isopropanol 80:20 at 2 mL/min, monitor at UV 254 and collect the vitamin D fraction at 6.0-7.5 min, evaporate the eluate to dryness under a stream of nitrogen. Reconstitute with 10 µL hexane:isopropanol 95:5 and inject on to a 50 × 4.6 Polygosil (Macherey-Nagel) + 250 × 4.6 7 µm Nucleosil 50-7 silica column, elute with hexane:isopropanol 85:5 at 2 mL/min, monitor the effluent at UV 254 and

collect the vitamin D fraction at 4.5 min, evaporate the vitamin D fraction, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 30-40 μm pellicular C18 (Vydac)
Column: 250 × 4.6 5 μm TP C18 (Vydac)
Mobile phase: MeOH
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Extracted: vitamin D3

KEY WORDS
serum; normal phase; reverse phase

REFERENCE
Parviainen,M.T.; Savolainen,K.E.; Korhonen,P.H.; Alhava,E.M.; Visakorpi,J.K. An improved method for routine determination of vitamin D and its hydroxylated metabolites in serum from children and adults, *Clin.Chim.Acta*, **1981**, *114*, 233–247.

SAMPLE
Matrix: formula
Sample preparation: Condition a 2.8 mL 500 mg silica SPE cartridge with 4 mL dichloromethane:isopropanol 80:20 and 5 mL dichloromethane:isopropanol 99.8:0.2. 15 mL Formula + 4 mL 46 ng/mL vitamin D_3 in EtOH + 15 mL ethanolic KOH, shake at 60° for 30 min, cool to room temperature, add 15 mL water, add 60 mL hexane, shake vigorously for 1.5 min, let stand for 10 min, discard aqueous layer. Wash the hexane layer with 15 mL water, add 15 mL water and 1 drop phenolphthalein to the hexane layer, add 10% acetic acid dropwise with shaking until the aqueous layer is colorless. Filter the hexane layer through anhydrous sodium sulfate, wash through with a few mL hexane, evaporate to dryness under reduced pressure at 40°, reconstitute with 2 mL dichloromethane:isopropanol 99.8:0.2, add to the SPE cartridge, rinse flask with 1 mL dichloromethane:isopropanol 99.8:0.2, add rinse to cartridge, wash with 2 mL dichloromethane:isopropanol 99.8:0.2, elute with 7 mL dichloromethane:isopropanol 99.8:0.2. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL MeCN, inject an aliquot. (Prepare ethanolic KOH by dissolving 140 g KOH in 310 mL EtOH, add 50 mL water. Prepare fresh each day.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 (not end-capped)
Mobile phase: Gradient. MeCN:MeOH:ethyl acetate 91:9:0 for 28 min, to 0:0:100 over 0.5 min, maintain at 0:0:100 for 2.5 min, return to initial conditions over 0.5 min, re-equilibrate for 2.5 min.
Column temperature: 27
Flow rate: 0.7 for 28 min, to 2.5 over 0.5 min, maintain at 2.5 for 4.5 min, return to initial conditions over 1 min
Injection volume: 250
Detector: UV 265

CHROMATOGRAM
Retention time: 19.5
Internal standard: vitamin D_3 (23)

KEY WORDS
protect from light and oxygen; SPE

REFERENCE
Sliva,M.G.; Sanders,J.K. Vitamin D in infant formula and enteral products by liquid chromatography: Collaborative study, *J.AOAC Int.*, **1996**, *79*, 73–80.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out powder equivalent to 200 IU vitamin D3, add 5 μg vitamin D2 and 10 mL EtOH:water 50:50. Extract with 15 mL hexane 3 times. Remove the organic layer and dry it under reduced pressure. Dissolve the residue in 1 mL MeOH:water 90:10 and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm TSK-gel ODS 80TM (TOSOH, Japan)
Mobile phase: MeOH:water 90:10
Flow rate: 1
Injection volume: 20
Detector: MS, Hitachi M-1000, APCI interface, drift voltage 20 V, focus voltage 120 V, vaporizer 399°, desolvation chamber 200°, multiplier voltage 2 kV

CHROMATOGRAM
Retention time: 14.4
Internal standard: vitamin D2
Limit of detection: 400 pg

OTHER SUBSTANCES
Simultaneous: vitamin D3

KEY WORDS
tablets; vitamin D2 is IS

REFERENCE
Adachi,T.; Nishio,M.; Yunoki,N.; Hayashi,H. Determination of vitamin D_3 and D_2 in multi-vitamin tablets by high-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry, *Anal.Sci.*, **1994**, *10*, 457–460.

SAMPLE
Matrix: formulations
Sample preparation: Tablets, capsules. Add 5 crushed tablets or the contents from 5 capsules to 10 mL DMSO, add 15 mL hexane, shake at 60° for 45 min, centrifuge at 3000 rpm for 10 min, remove hexane layer, add 15 mL hexane, vortex for 5 min at room temperature, remove hexane layer, repeat hexane extraction three more times, combine all hexane layers, filter, make up to 100 mL with hexane, dilute if necessary, inject a 100 μL aliquot. Syrup. 10 mL Syrup + 10 mL DMSO + 15 mL ether:hexane 10:90, vortex for 5 min, remove hexane layer, repeat extraction four more times, combine the extracts, evaporate with nitrogen until the ether is removed, filter, make up to 100 mL with hexane, dilute if necessary, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm amino bonded phase (Chromatography Sciences Co.)
Mobile phase: Hexane:isopropanol 99:1
Flow rate: 1-2
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: vitamin A
Interfering: cholecalciferol (vitamin D3)

KEY WORDS
work under subdued light; tablets; capsules; syrup

REFERENCE
Beaulieu,N.; Curran,N.M.; Gagné,C.; Gravelle,M.; Lovering,E.G. Liquid chromatographic methods for vitamins A and D in multivitamin-mineral formulations, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 247–254.

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20min. Extract the vitamins twice with 10 mL n-hexane. Evaporate to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5μm C18 (Alltech, CA, USA)
Mobile phase: MeOH:EtOH 80:20 (A) or EtOH:water 95:5 (B)
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM
Retention time: 8 (A), 9 (B)

OTHER SUBSTANCES
Extracted: isotretinoin, retinal, tretinoin, vitamin A, vitamin D3, vitamin E, vitamin K1, vitamin K2

REFERENCE
Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE
Matrix: solutions
Sample preparation: Make up a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax PRO-10 C18
Mobile phase: MeOH:MeCN:hexane 95:3:2
Flow rate: 1
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: cholecalciferol (vitamin D3), tachysterol

KEY WORDS
keep at 4° away from light; also preparative details

REFERENCE
Letter,W.S. Preparative isolation of vitamin D2 from previtamin D2 by recycle high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *590*, 169–173.